

Claims

1. A method for trapping reaction intermediates of an oxidoreductase comprising the steps of:

5 (the first step) dissolving an oxidoreductase, a photoinduced reducing agent that releases electrons by light-irradiation, amine-type electron donor and a substrate for said oxidoreductase in water and mixing these;

(the second step) cooling the mixture prepared in the first step to 70~270K to be frozen;

10 (the third step) irradiating the frozen mixture prepared in the second step at 70~270K with a light in a wavelength region including the absorbing wavelength of said photoinduced reducing agent; and

(the fourth step) raising the temperature of the frozen mixture prepared in the third step to the temperature that is 80~270K and is higher than the temperature of the
15 third step.

2. The method as in claim 1 wherein the mixture prepared in the first step is cooled to the temperature lower than the temperature (hereinafter referred to as "diffusion onset temperature") at which the substrate starts to diffuse in said mixture in the
20 second step; the frozen mixture prepared in the second step is irradiated with a light at a temperature lower than the diffusion onset temperature in the third step; and the temperature of the frozen mixture prepared in the third step is raised to a temperature higher than the diffusion onset temperature but lower than 270K in the fourth step.

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3. The method as in claim 2 wherein the frozen mixture prepared in the second step is irradiated with a light at a temperature which is 5~20K lower than the diffusion onset temperature in the third step; and the temperature of the frozen mixture prepared in the third step is raised to a temperature between the diffusion onset
30 temperature and the diffusion onset temperature plus 50K but lower than 270K in the fourth step,.

4. The method as in any one of claims 1 to 3 further comprising (fifth step) cooling the frozen mixture prepared in the fourth step to a temperature lower than the diffusion onset temperature.